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Functional polymorphisms of HSPA5: Possible association with bipolar disorder

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Abstract

Altered endoplasmic reticulum stress (ER) response signaling is suggested in bipolar disorder. Previously, we preliminarily reported the genetic association of *HSPA5* (*GRP78*/*BiP*) with bipolar disorder. Here, we extended our analysis by increasing the number of Japanese case-control samples and NIMH Genetics Initiative bipolar trio samples (NIMH trios), and also analyzed schizophrenia samples. In Japanese, nominally significant association of one haplotype was observed in extended samples of bipolar disorder but not in schizophrenia. In NIMH trios, no association was found in total samples. However, an exploratory analysis suggested that the other haplotype was significantly over-transmitted to probands only from the paternal side. The associated haplotype in Japanese or NIMH pedigrees shared three common polymorphisms in the promotor, which was found to alter promotor activity. These findings suggested promotor polymorphisms of HSPA5 may affect the interindividual variability of ER stress response and may confer a genetic risk factor for bipolar disorder.

Keywords: Bipolar disorder; Schizophrenia; Endoplasmic reticulum stress; HSPA5/GRP78; Association study; Promotor assay

Bipolar disorder is a severe mental disorder characterized by recurrent episodes of mania and depression, affecting about 0.5–1% of the population [1]. Although the contribution of genetic factors has been evidenced by family, twin and adoption studies, the molecular pathophysiology of the illness has been controversial [2,3]. Recently, we suggested that the endoplasmic reticulum (ER) stress response signaling is one of candidate cascades related to pathology of the illness [4].

In our previous study, *XBP1* and *HSPA5* were down-regulated in the lymphoblastoid cells of monozygotic twins with bipolar disorder compared with healthy co-twins by DNA microarray analysis. Induction of *XBP1* and *HSPA5* mRNA by thapsigargin was reduced in the patients' cell lines and valproate induced *ATF6* mRNA expression and enhanced the ER stress response in SHSY5Y cells [4]. Although we also reported that a functional polymorphism of *XBP1* (−116C/G) altering the ER stress response was

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associated with bipolar disorder, the genetic association was not replicated in Caucasian bipolar samples and Taiwanese samples [5,6]. On the other hand, the association of *XBP1* −116C/G polymorphisms with schizophrenia was observed in Chinese samples and Japanese samples [7,8]. Schizophrenia is another major mental disorder sharing common clinical features and genetic background with bipolar disorder [9]. The chromosomal region of *XBP1*, 22q, is one of common linkage loci for these two disorders. Thus, altered ER stress response signaling may contribute to the pathophysiology of both of these major mental disorders.

When unfolded proteins accumulate in endoplasmic reticulum (ER) by some reasons, ER stress response begins. ER stress response consists of four signaling cascades: (1) induction of ER chaperon such as *HSPA5* (*GRP78*/*BiP*), which promotes the folding of unfolded proteins (unfolded protein response, UPR), (2) inhibition of protein synthesis, (3) induction of ER-associated degradation pathway, which promotes the processing of unfolded proteins, and (4) induction of apoptosis when this system could not process the unfolded proteins [10,11].

In the previous paper, we focused on *XBP1* since it is a transcription factor regulating the mRNA expression of ER chaperon genes such as *HSPA5*. However, initial reaction eliciting ER stress response is the consumption of HSPA5. When HSPA5 proteins are consumed to fold unfolded proteins, dissociation of HSPA5 from ATF6 protein on the ER membrane causes cleavage of ATF6. Cleaved ATF6 protein induces the expression of ER chaperons and *XBP1*. In parallel, dissociation of HSPA5 from IRE1 protein on the ER membrane causes dimerization of IRE1, which splices *XBP1* mRNA. The spliced *XBP1* mRNA encodes active form XBP1 that strongly induces the expression of chaperon genes including *HSPA5* as well as *XBP1* itself [10]. In this regard, HSPA5 is a key protein regulating ER stress response.

HSPA5, *TRA1* (*GRP94*), and *CALR* (*Calreticulin*) are known to be increased in the temporal cortex of depressed subjects who died by suicide [12]. Anti-malarial drug mefloquine, which is known to cause psychiatric symptoms including bipolar disorder in susceptible individuals [13], is reported to induce ER chaperons including HSPA5 in rat neurons [14]. Methamphetamine (MAP), a psychostimulant causing manic state, is also known to induce *HSPA5* and other ER chaperon genes in the mouse brain [15]. Induction of *HSPA5* by mefloquine or MAP is interpreted that these drugs cause ER stress, since they also induce ER stress pathway other than UPR, such as apoptosis. On the other hand, valproate, one of the mood stabilizers, is known to increase *HSPA5* expression and have neuroprotective effects by enhancing the UPR [16–20,4]. Recently, the other mood stabilizer, lithium, was also shown to protect the rat PC12 cells against ER stress by inducing the *HSPA5* mRNA [21].

HSPA5 gene is located on 9q33–34.1, on which significant evidence for linkage with bipolar disorder was observed by several studies [22–24].

In this study, we examined whether or not genetic variations of *HSPA5* contribute to the pathophysiology of bipolar disorder and schizophrenia.

Part of the data presented in this paper (data on 3 of 6 SNPs in 195 of 439 patients with bipolar disorder and 254 of 492 controls in case-control studies, and 88 of 240 trios in transmission disequilibrium test) was reported in the reply to correspondence [5].

Materials and methods

Subjects. For the case-control study, 439 patients with bipolar disorder (50.5 ± 13.4 years old, 208 males and 231 females), 229 patients with schizophrenia (46.0 ± 14.9 years old, 131 males and 98 females), and 492 controls (41.7 ± 14.4 years old, 246 males and 246 females) were analyzed. In addition to the samples previously reported in the reply to correspondence [5], we increased the number of case-control samples including the independently collected sample set described previously as "MPS samples" for replication study [25]. MPS samples include 239 patients with bipolar disorder (51.0 ± 13.1 years old, 131 males and 108 females) and 234 controls (51.6 ± 10.7 years old, 117 males and 117 females). They were diagnosed according to the DSM-IV criteria (American Psychiatric Association). Controls were selected from students, nurses, office workers, and doctors in participating institutes, and their friends. A senior psychiatrist interviewed them and they did not have major mental disorders. Only a part of them were interviewed using a structured interview, Mini-International Neuropsychiatric Interview (M.I.N.I.) [26]. In Japanese, no significant population stratification has repeatedly reported in several studies including a part of our samples [4,27–29]. For transmission disequilibrium test, we analyzed total 240 trio samples (227 trios with BPI proband and 12 trios with BPII proband) from NIMH Genetics Initiative Pedigrees (http://zork.wustl.edu/nimh/), including 88 trios previously reported in the reply to correspondence [5]. Only one trio was obtained from one family. The criteria, by which the trio was selected from a pedigree, were, (1) DNA is available for parents and the proband, (2) if multiple complete trios were found in one pedigree, the trio with younger generation was selected, (3) if multiple trios were available in one generation, elder sibling was selected as the proband. Data and biomaterials of the NIMH pedigrees were collected in four projects that participated in the NIMH Bipolar Disorder Genetics Initiative. From 1991 to 1998, the Principal Investigators and Co-Investigators were: Indiana University, Indianapolis, IN, U01 MH46282, J. Nurnberger, M. Miller, and E. Bowman; Washington University, St. Louis, MO, U01 MH46280, T. Reich, A. Goate, and J. Rice; Johns Hopkins University, Baltimore, MD, U01 MH46274, J. R. DePaulo, Jr., S. Simpson, and C. Stine; NIMH Intramural Research Program, Clinical Neurogenetics Branch, Bethesda, MD, E. Gershon, D. Kazuba, and E. Maxwell. Written informed consent was obtained from all the subjects. Postmortem brains were donated by The Stanley Medical Research Institute's Brain Collection courtesy of Drs. Michael B. Knable, E. Fuller Torrey, Maree J. Webster, Serge Weis, and Robert H. Yolken. The Ethics Committees of the Brain Science Institute (RIKEN) and participating institutes approved the study.

Mutation screening of the HSPA5 genes and genotyping of flag SNPs. Polymorphisms of all exons and the upstream region (1 kb) of *HSPA5* (GenBank Accession No. NT_008470) were screened by sequencing in 24 patients with bipolar disorder and eight patients with schizophrenia. Primer sequences are available on request. Genotyping was performed using commercially available TaqMan probes and ABI7900HT according to the protocol recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA).